ABSTRACT

To unravel the mysteries of complex biological processes carried out by biomolecules it is necessary to adopt a multifaceted approach, which involves employing a wide variety of tools both computational and experimental. In order to gain a clear understanding of the function of biomolecules their three dimensional structure is required. X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy are the only two methods capable of providing high-resolution three-dimensional structure of biomolecules. NMR has the advantage of allowing the study of structure of biomolecules in solution and is better equipped to characterize the dynamics of the protein. Protein structure determination by NMR spectroscopy consists of recombinant expression of isotopically labeled proteins, purification, data collection, data processing, resonance assignment, distance restraint and angular restraint generation, structure calculation and structure validation. Apart from 3D structure determination of biomolecules NMR has become the method of choice for studying transient protein-protein interactions, which are notoriously difficult to study at higher resolution by other methods.

Mass spectrometry plays an important role in enabling rapid identification of biomolecules and their modifications. The high sensitivity and resolution mass spectrometry offers makes it the method of choice for studying post-transitional modification of proteins.

Use of computers in biology has played an essential role in elucidating those structure function relationships in biomolecules that are not possible to study by experimental techniques.

The first chapter of this thesis deals with the introduction of methods used in this study. A brief introduction about the theory of Nuclear Magnetic Resonance (NMR) spectroscopy is given. Protein NMR methods used for structure determination of medium sized proteins are discussed. A part of this chapter discusses about the application of mass spectrometry in biochemistry and the use of tandem MS/MS experiments in identification of proteins and peptide fragments. Finally, the last part of this chapter gives an introduction about the theory of molecular dynamics and techniques used in the post processing of MD trajectories to elucidate the dynamics of proteins.

The second chapter of this thesis is concerned with NMR characterization of a novel protein-protein interaction between the glycolytic enzyme Triosephosphate isomerase and the redox protein Thioredoxin. Chemical shift perturbation studies have been done to map the binding interfaces of these proteins. The structure of the complex was then modeled using NMR restraints based docking using the known 3D structure of these proteins. The docked complex reveals crucial insights into the glutathione mediated redox regulation of Triosephosphate isomerase and the role of thioredoxin as a deglutathionylating agent. Enzyme activity assays of Triosephosphate isomerase were done to show the inhibitory effects of s-glutathionylation of Cys217 and the role of thioredoxin as a deglutathionylating agent.

The third chapter of the thesis is aimed to address some important issues related to the inhibition of Plasmodium falciparum Triosephosphate isomerase by S-glutathionylation. Oxidative stress induces protein glutathionylation which is a reversible post translational modification consisting of the formation of a mixed disulfide between protein cysteines and glutathione. Mass spectrometric analysis of the kilnetics of glutathionylation along

with enzyme activity assays clearly show that gluthionylation of either Cys-13 (situated in the dimmer interface) or Cys-217 (situated in Helix G) can render the enzyme inactive. Molecular dynamics simulations provide a mechanistic basis of inhibition and predict that glutathionylation at Cys217 allosterically induces loop 6 disorder.

The fourth chapter of this thesis addresses the stabilizing effect of introduction of a cross-strand disulfide bond across a non-hydrogen bonded position of an antiparallel beta sheet. Multidimensional heteronuclear NMR experiments have been used to get the backbone and side-chain resonance assignments, distance and angular restraints. In addition RDC based restraints have been used to calculate the structure of oxidixed form of L79C, T89C thiroedoxin. The observation of predominantly –RH staple conformation among the NMR ensemble in typical of cross-strand disulfides.

The fifth chapter of this thesis deals with the dynamics of thioredoxin using computational methods.In this chapter analysis of known complexes of thiroedoxin was done to determine binding hot spot residues using free energy calculations. The physicochemical basis for the multispecificity of thioredoxin is probed using molecular dynamics simulations. In this chapter it has been shown that conformational selection plays a very important role in thioredoxin target recognition.